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Quantitative microbiological slaughter process analysis in a large-scale Swiss poultry abattoir

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Abstract

We performed a quantitative microbiological slaughter process analysis in a large-scale Swiss poultry abattoir. At each of six selected process steps (plucking, cloaca excision, evisceration, washing, water spray chiller and dry air chiller), 103 carcasses from 50 flocks were sampled and quantitatively tested for indicator bacteria (total viable counts, *Escherichia (E.) coli*/*Enterobacteriaceae* counts) as well as for *Campylobacter* spp. and extended-spectrum beta-lactamase-producing *Enterobacteriaceae*. Samples consisted of a pooled back, breast and leg skin sample as well as swab samples from the thoracic and abdominal cavities. On skin samples, the presence of *E. coli* and *Enterobacteriaceae* increased between plucking and evisceration (on average by 1.2 log CFU/g), while washing decreased the microbial load (on average by 0.8 log CFU/g). Subsequent chilling slightly reduced counts on carcasses skin and markedly reduced the counts in the abdominal cavity. ESBL-producing *E. coli* was present above the limit of detection (LOD) with a prevalence of 6.1%, the majority of which belonged to phylogenetic group A. The $bla_{\text{CTXM-1}}$ gene was found in all ESBL producing strains. The prevalence of *Campylobacter* above the LOD was 16.7%, and none of the final, chilled carcasses had counts that exceeded 1000 CFU/g. Multilocus sequence typing of 120 *Campylobacter jejuni* strains yielded 15 different sequence types (ST) among which ST 21 was predominant with 31.7% and four newly defined ST.

Keywords: Broiler carcass, slaughter process, *Campylobacter*, *Escherichia coli*, *Enterobacteriaceae*, ESBL

Zusammenfassung

Im Rahmen dieser Arbeit wurde eine mikrobiologische Schlachtprozessanalyse in einem Schweizer Geflügelschlachtbetrieb durchgeführt. An sechs Prozessstufen (Rupfen, Kloakenschneider, Eviszerator, Dusche, Wasserkühler, Trockenkühler) wurden 103 Schlachttierkörper (STK) aus 50 Geflügelherden quantitativ auf Indikatorbakterien (Gesamtkeimzahl, *Escherichia (E.) coli*/*Enterobacteriaceae*), *Campylobacter* spp. und Extended-Spektrum-Betalaktamasen (ESBL) produzierende *Enterobacteriaceae* getestet. Von jedem STK wurde eine Poolprobe, bestehend aus Rücken-, Brust- und Schenkelhaut untersucht, sowie Tupferproben von definierten Flächen aus Thorax- und Abdominalhöhle. Von der Prozessstufe «Rupfen» bis «Eviszeration» stieg die Kontamination mit *E. coli* und *Enterobacteriaceae* bei den Hautproben an (im Durchschnitt 1.2 log KBE/g), während das Abduschen der STK den Keimgehalt senkte (im Durchschnitt 0.8 log KBE/g). Das Kühlen der STK hatte einen reduzierenden Einfluss auf den Keimgehalt der Haut und der Abdominalhöhle. ESBL-bildende *E. coli* waren mit einer Prävalenz von 6.1% nachweisbar und die Mehrheit der gefundenen Stämme gehörte zur phylogenetischen Gruppe A. Das $bla_{\text{CTXM-1}}$ Gen war bei allen ESBL-Bildnern vorhanden. Die Prävalenz von *Campylobacter* war 16.7%. Am Ende der Kühlkette wies keiner der untersuchten STK Zahlen von >1000 KBE/g auf. Die Multi Locus Sequenz Typisierung von 120 *Campylobacter jejuni* Stämmen ergab 15 verschiedene Sequenztypen (ST), wobei ST 21 am häufigsten gefunden wurde.

Schlüsselwörter: Geflügelschlachttierkörper, Schlachtprozess, *Campylobacter*, *Escherichia coli*, *Enterobacteriaceae*, ESBL-Bildner

Quantitative microbiological slaughter process analysis in a large-scale Swiss poultry abattoir

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Abstract

We performed a quantitative microbiological slaughter process analysis in a large-scale Swiss poultry abattoir. At each of six selected process steps (after plucking, cloaca excision, evisceration, washing, water spray chiller and dry air chiller), 103 carcasses from 50 flocks were sampled and quantitatively tested for indicator bacteria (total viable counts, *Escherichia coli*/*Enterobacteriaceae* counts) as well as for *Campylobacter* spp. and extended-spectrum beta-lactamase-producing *Enterobacteriaceae*. Samples consisted of a pooled back, breast and leg skin sample as well as swab samples from the thoracic and abdominal cavities. On skin samples, the presence of *Escherichia coli* and *Enterobacteriaceae* as indicators of fecal contamination increased between plucking and evisceration (on average by 1.2 log CFU/g), while washing after evisceration successfully decreased the microbial load (on average by 0.8 log CFU/g). Subsequent chilling slightly reduced microbial counts on carcasses skin and markedly reduced the counts in the abdominal cavity. ESBL-producing *Escherichia coli* was present above the limit of detection (LOD) with a prevalence of 6.1%, the majority of which belonged to phylogenetic group A. The $bla_{\text{CTXM-1}}$ gene was found in all ESBL producing strains. The prevalence of *Campylobacter* above the LOD was 16.7%, and none of the final, chilled carcasses had counts that exceeded 1000 CFU/g. Multilocus sequence typing of 120 *Campylobacter jejuni* strains yielded 15 different sequence types (ST) among which ST 21 was predominant with 31.7% and four newly defined ST.

1. Introduction

The growing demand for poultry meat worldwide (FAO, 2019) leads to an ever-increasing production volume in highly automated poultry abattoirs, with 2.3 million tons of broiler carcass weight produced in the EU in 2016 (EFSA Panel on Biological Hazards (EFSA BIOHAZ Panel) et al., 2019). Human pathogens such as *Campylobacter* are frequently isolated from the gastrointestinal tract of healthy poultry (Oakley et al., 2014) and pose a risk to consumers when they are transmitted to the carcass via fecal contamination during the slaughter process. Additionally, multiresistant strains of *Campylobacter* and extended spectrum betalactamase (ESBL)-producing strains of *E. coli* and *Salmonella* are isolated from the poultry environment with increasing frequency (Kaakoush, Castaño-Rodríguez, Mitchell, & Man, 2015; Saliu, Vahjen, & Zentek, 2017). *Campylobacter jejuni* and, to a lesser extent, *C. coli,* are the leading causes of human gastroenteritis in the EU (European Food Safety Authority and European Centre for Disease Prevention and Control (EFSA and ECDC), 2018) and worldwide (Kaakoush et al., 2015). Of those, an estimated 50-80% of all human cases of campylobacteriosis are attributable to the chicken reservoir (EFSA Panel on Biological Hazards (BIOHAZ), 2010). The European Food Safety Agency EFSA estimates that the risk to human health through consumption of broiler meat could be reduced by 50% if broiler carcasses do not exceed <1000 CFU/g (EFSA Panel on Biological Hazards (BIOHAZ), 2011), which lead to the introduction of a new process hygiene criterium for poultry meat in the EU in 2018 (Commission Regulation (EU) 2017/1495 of 23 August 2017). Compliance with increasingly strict regulations, the control of food borne pathogens as well as the adherence to physical and sensory quality requirements are a major focus of quality control efforts in the industry, and large-scale poultry abattoirs are investing considerably into food safety to avoid recalls and to protect their reputation (Viator, Muth, Brophy, & Noyes, 2017). The FDA estimates that the development of food safety plans such

as Sanitation Standard Operating Procedures (SSOP) or Hazard Analysis and Critical Control Point (HACCP) costs food business operators between $6000 and $87000 (Viator et al., 2017). Thorough process analyses are needed to make informed decisions to invest in areas of the of the highly automated poultry slaughter process that will influence contamination of poultry carcasses with potentially harmful human pathogens most.

We conducted a quantitative microbial slaughter process analysis of broiler carcasses in a large-scale Swiss poultry abattoir to determine the total microbial load at six different processing steps. Unlike previous research (Berrang & Dickens, 2000; Pacholewicz, Swart, et al., 2015b; Zweifel, Althaus, & Stephan, 2015), the cloaca excision step between plucking and evisceration was individually investigated, and the thorax and abdominal cavities were sampled in conjunction with skin samples. In addition to quantitative data on total viable counts and fecal contaminants such as *Enterobacteriaceae* and *Escherichia coli*, *Campylobacter* and ESBL-producing *E. coli* were quantified.

2. Materials and Methods

2.1. Sampling

All samples originated from a Swiss poultry abattoir that ran at a line speed of 9500 animals per hour, resulting in a total of approximately 70000 animals per day. Samples from broiler carcasses were taken at six different slaughter process steps: after plucking, after cloaca excision, after evisceration, after washing, after chiller 1 (air chiller with water spray, -0.7 °C, 45min) and after chiller 2 (dry air chiller, +0.2 °C, 120min). At each process step, 103 broiler carcasses were sampled between February and June 2018. A total of 618 carcasses originating from 50 different flocks were tested. To determine if the microbial quality of the product deteriorated throughout the day, samples were alternatively taken at the beginning (morning), in the middle (midday) and towards the end (afternoon) of the workday.

At each process step, skin samples from the breast, back and leg were obtained and pooled. The exact areas to be sampled were defined after a risk-based process analysis. Two additional swab samples, one of a defined area in the abdominal cavity (10 cm^2) and one of a defined area in the thoracic cavity (10 cm^2), were taken after evisceration, after washing, and after chillers 1 and 2. Samples were placed in sterile bags and cooled immediately. All samples were transported to the laboratory under preservation of the cold chain and analyzed the next day.

The 50 flocks included in this study belonged to three different categories, comprising the following production labels: free-range (114/618, 18.4%), organic (96/618, 15.5%), or conventional (408/618, 66.0%). In the conventional label animals were kept indoor as well as in a closed winter garden. Broilers in the free-range label additionally had access to a pasture, while animals raised under the organic label were housed in small units of 500 animals with access to pasture 24/7. The number of tested carcasses per label was chosen to reflect the proportion of these labels in the routine slaughtering process in this particular abattoir. Broilers from the conventional production label were on average 33 days old at slaughter, those from the free-range label 57 days, and those from the organic label 64 days.

2.2. Microbiological examination

A quantitative microbial analysis of the carcass samples was obtained by enumerating total viable counts (TVC), *Escherichia (E.) coli*, *Enterobacteriaceae*, *Campylobacter* spp. and extended spectrum beta-lactamases (ESBL) producing *Enterobacteriaceae*. The skin samples were diluted 1:10 weight/weight in 0.85 % saline, the swab samples were diluted by adding 10 mL of 0.85 % saline. All samples were homogenized in a Seward Stomacher 400 (VWR, Dietikon, Switzerland) for 30 s. Colony forming units (CFU) were determined by direct colony count on the following agars: Plate Count agar (PC, Oxoid AG, Pratteln, CH; 72 h, 30°C), Rapid *E. coli* agar (Bio-Rad Laboratories AG, Reinach, CH; 24 h, 37°C), Violet Red

Bile Glucose agar (VRBG, Becton Dickson AG, Allschwil, CH; 48h, 37°C, anaerobic conditions), CampyFood agar (bioMérieux SA, Geneva, CH; 48h, 41.5°C, microaerophilic conditions), chromogenic Brilliance ESBL agar (Oxoid; 24h, 37°C). Results were expressed as mean log CFU/g for skin samples and mean log CFU/cm^2 for swab samples. Due to the different methods used for skin (pooled, cut skin) and cavity samples (swabbing of a defined area), the limit of detection (LOD) was 100 CFU/g for skin samples, and 10 CFU/cm^2 for swab samples.

2.3. Species identification and phylogenetic typing

Presumptive *Campylobacter* and ESBL isolates were identified by MALDI-TOF MS. Material from a single colony was transferred to a MALDI target plate, overlaid with 1µl formic acid followed by 1ul α-Cyano-4-hydroxycinnamic acid (HCCA) and read on a Bruker Microflex LT/SH (Bruker, Fällanden, Switzerland). Confirmed *Campylobacter jejuni* isolates were subtyped by multi-locus sequence typing (MLST), and confirmed ESBL producers were further analyzed for the presence of specific genes coding for β-lactamases. DNA was purified using a standard heat lysis protocol (Russell & Sambrook, 2006). All PCR products were purified using the GenElute™ PCR Clean-Up (Sigma-Aldrich, Buchs, Switzerland) according to the manufacturer's recommendations.

For *C. jejuni*, internal fragments of seven housekeeping genes (*aspA, glnA, gltA, glyA, pgm, tkt* and *uncA*) were amplified. PCR products were sequenced and allele numbers and sequence types (STs) were assigned using the *Campylobacter* PubMLST database (https://pubmlst.org/*Campylobacter*/, accessed November 10th, 2018). See supplementary table 1 for the primer sequences.

All ESBL producers were tested for the presence of five genes belonging to the bla_{TEM}, bla_{SHV} (Pitout et al., 1998) and bla_{CTX-M} (Geser, Stephan, & Hächler, 2012)families, and the nucleotide- and translated protein-sequences were analyzed with CLC Main Workbench 7.0.2

(CLC bio, Aarhus, Denmark). For database searches the BLASTN program of NCBI (http://www.ncbi.nlm.nih.gov/blast/, accessed November 2018) was used.

2.4. Antimicrobial susceptibility testing

Susceptibility against antibiotics was determined by using the disc diffusion method according to the EUCAST protocol and evaluated according to EUCAST criteria (http://www.eucast.org/clinical_breakpoints/, accessed November 2018).

Isolates of *C. jejuni* were tested for susceptibility to ciprofloxacin (CIP5), erythromycin (E15) and tetracycline (TE30) (Becton, Dickinson, Heidelberg, Germany). ESBL producing *E. coli* isolates were tested for susceptibility to ampicillin (AM10), amoxicillin-clavulanic acid (AMC30), azithromycin (AZM15), cefazolin (CZ30), cefepime (FEP30), cefotaxime (CTX30), chloramphenicol (C30), ciprofloxacin (CIP5), fosfomycin (FOS200), gentamicin (GM10), kanamycin (K30), nalidixic acid (NA30), nitrofurantoin (F/M300), streptomycin (S10), sulfamethoxazole with trimethoprim (SXT) and tetracycline (TE30) (Becton, Dickinson, Heidelberg, Germany).

2.5. Data analysis

Results were expressed log CFU values per g or cm^2. To avoid assuming that results below the LOD signify complete absence of the organism in question, and to be able to log transform, the values for samples with results below the LOD were set to 10 CFU/g. Statistical analysis was performed using RStudio 1.1.463. Linear mixed effects model using lmer in LmerTest (Kuznetsova, Bruun Brockhoff, & Bojesen Christensen, 2016) were calculated for TVC, *E. coli* and *Enterobacteriaceae* using station (e.g. after plucking, after cloaca excision, after evisceration, after washing, after chiller 1 and after chiller 2), location (e.g. skin, thorax or abdomen) as fixed effects and the chicken ID as random effect. Neither time of slaughter nor label (e.g. organic, free-range and conventional) explained a significant proportion of the variance in the data, and were excluded from the model based on AIC.

lsmeans (Lenth, 2016) was used to create contrasts. A chi-square test was performed to test the hypothesis that broilers grown under the conventional label harbored *Campylobacter* and ESBL less frequently than those from organic and free-range labels. The level of significance was set at α=0.05. All graphics were done using ggplot2 (Wickham, 2009). The full datasets and R scripts are available as supplementary data 1.

3. Results

Enterobacteriaceae above the limit of detection were found in 587 (95.0 %), *E. coli* in 572 (92.6 %), and *Campylobacter* spp. in 77 (12.5 %) of all broiler carcasses in at least one sample (supplementary data 1).

3.1. TVC, *E. coli* and *Enterobacteriaceae* on skin samples during slaughter process

While TVC were relatively stable during the slaughtering process, *Enterobacteriaceae* and *E. coli* counts on the skin were significantly increased after the cloaca excision step, with the following washing and cooling steps restoring them back to the original level (Figure 1).

After plucking, mean log TVC of skin samples of broiler carcasses measured 4.2 log CFU/g. The counts were slightly increased after the cloaca excision and evisceration process steps. This increase was not significant (p>0.05). Subsequent washing significantly reduced the counts from 4.4 log CFU/g to 4.1 log CFU/g, after which the counts remained constant through the remainder of the chilling process.

Counts for *E. coli* and *Enterobacteriaceae* of skin samples were comparable, indicating that the *Enterobacteriaceae* that were found were mainly *E. coli*. After plucking, mean counts for both *E. coli* and *Enterobacteriaceae* were 2.5 log CFU. After the cloaca excision step, the counts for *E. coli* and *Enterobacteriaceae* significantly increased to 3.7 log CFU/g and 3.6 log CFU/g, respectively. In the following evisceration step mean values for *E. coli* and *Enterobacteriaceae* remained constant, while washing significantly reduced the counts for

both *E. coli* and *Enterobacteriaceae* from 3.8 log CFU/g to 3.0 log CFU/g. During the subsequent chilling steps, mean values decreased by less than 0.5 log CFU/g, resulting in 2.6 log CFU/g for *E. coli* and 2.7 log CFU/g for *Enterobacteriaceae* in samples from the final product.

With regard to the time of slaughter (morning, midday, afternoon) and to carcasses originating from the three different labels, TVC, *E. coli* and *Enterobacteriaceae* counts on skin did not differ significantly (supplementary data 1).

3.2. TVC, *E. coli* and *Enterobacteriaceae* on abdominal and thoracic samples during the slaughter process

After evisceration, the abdominal and thoracic cavities were accessible for sampling and tested using swab samples. A decreasing trend in microbial counts can be observed in all samples after the washing and chilling steps, even though the microbial counts are not directly comparable due to the different methods used (CFU/g for skin samples, CFU/cm^2 for abdominal and thoracic swabs, lower LOD for swab samples). Microbial counts for TVC, *E. coli* and *Enterobacteriaceae* in the thorax samples were 0.5 - 1.0 log CFU/cm^2 lower compared to those in the abdominal cavity (Figure 2).

In the abdominal cavity of the broiler carcasses, the mean log TVC was 4.2 log CFU/cm^2 after evisceration. Washing as well as the two chilling steps significantly reduced TVC counts to 3.4 log CFU/cm^2 in the final product. The counts for *E. coli* and *Enterobacteriaceae* in the abdominal cavity were 3.6 log CFU/cm^2 after evisceration, which were significantly reduced to 2.7 and 2.8 CFU/cm^2 in the final product after washing and chilling.

In the thoracic cavity, TVC counts were 3.7 log CFU/cm^2 after evisceration with a significant reduction to 3.4 CFU/cm^2 after washing and 3.1 log CFU/cm^2 in the final product. Counts for *E. coli* and *Enterobacteriaceae* were at 2.6 log CFU/cm^2 after evisceration and showed a

steady, statistically non-significant decrease to 2.2 and 2.3 log CFU/cm^2, respectively in the final product.

As with the skin samples, neither the time of slaughter nor the label a carcass originated from significantly influenced the microbial counts in the abdominal or thoracic cavities (supplementary data 1).

3.3. Frequency and characterization of *Campylobacter* spp. on chicken carcasses

One hundred and three (103, 16.7%) out of 618 poultry carcasses showed *Campylobacter* counts above the LOD (Figure 3a). Free-range (49, 43.0%) and organic (23, 24.0%) broiler carcasses were more often contaminated with *Campylobacter* above the LOD compared to conventionally kept broilers (31, 7.6%) ($p<0.001$). From the plucking to the evisceration step, *Campylobacter* counts were above 1000 CFU/g in 18 broiler carcasses, whereas none of the samples obtained after the washing or chilling steps exceeded 1000 CFU/g.

From the 103 *Campylobacter*-positive carcasses, 167 *Campylobacter* strains were isolated, of which 147 were identified as *C. jejuni* and 20 as *Campylobacter coli* by MALDI-TOF MS. One hundred and twenty *C. jejuni* strains were chosen for MLST analysis and antimicrobial susceptibility testing. The following inclusion criteria were applied: at least one *C. jejuni* isolate from each *Campylobacter*-positive carcass was selected and if possible, a skin isolate as well as an isolate from either the abdominal or the thoracic cavity of each carcass were included.

The detected STs of *C. jejuni* isolates across the six different slaughter process steps are summarized in Table 1. A total of 15 STs, including four novel types (9454, 9455, 9456 and 9457), were observed among the 120 isolates. ST 21 was the most frequent type with 31.7%, followed by ST 48 (14.2%) and ST 51 (11.7%). Strains sequenced from skin and body cavity samples of the same carcass yielded the same ST in 92.6%.

55 (45.8%) out of 120 tested *C. jejuni* strains were resistant to ciprofloxacin and tetracycline. In addition, eight (6.7%) strains were resistant only to ciprofloxacin, one (0.8%) strain only to tetracycline and three (2.5%) strains to erythromycin. Antimicrobial resistance patterns could not be associated with specific STs.

3.4. Frequency and characterization of ESBL isolates on chicken carcasses

38 (6.1%) out of the 618 tested samples showed growth of ESBL producing *E. coli* above the LOD on chromogenic Brilliance ESBL agar. A comparable proportion of conventional (7.6 %) and free-range (6.1 %) broiler carcasses harbored ESBL, while no ESBL was detected in carcasses originating from organic label farms (Figure 3b). From the 38 ESBL-positive carcasses, 51 strains were isolated and identified as *E. coli* by MALDI-TOF. All isolates were further typed by antimicrobial susceptibility testing, phylogenetic typing and identification of ESBL gene variant (Supplementary table 2).

All 51 tested *E. coli* isolates were resistant to ampicillin and cefazolin, and 50 (96.2%) harbored resistance to cefotaxime. Furthermore, 38 strains (73.1%) were resistant to nalidixic acid. Genetic analysis revealed that the dominant resistance gene was $bla_{\text{CTX-M-1}}$ (82.4%), followed by the combination of $bla_{\text{CTX-M-1}}$ and $bla_{\text{TEM-1b}}$ (11.8%) and the combination of $bla_{\text{CTX-M-1}}$ and bla_{SHV12} (5.9%). Most of the 51 isolated *E. coli* strains belonged to phylogenetic group A (92.2%).

4. Discussion

The detailed, stepwise analysis of the slaughter process performed here identified the process steps between plucking and evisceration, and washing with cold water after evisceration as the two key steps that influenced microbial counts on the broiler carcasses. To our knowledge, no previous studies differentiate the evisceration step into its components of cloaca excision and evisceration. The main source of fecal contamination on the carcass

surface was between plucking and evisceration in this slaughterhouse, as indicated by the increase of *Enterobacteriaceae* and *E. coli* by more than one order of magnitude after cloaca excision, while the subsequent evisceration had a negligible effect on bacterial counts. Electrostimulation between plucking and cloaca excision may have caused fecal matter to be expelled from the carcass, leading to surface contamination.

Washing with cold water efficiently reduced TVC as well as counts of fecal contaminants. This was in agreement with other studies that reported a decrease in *E. coli* and *Enterobacteriaceae* after washing, regardless of whether chlorine was added to the water (Berrang & Bailey, 2009; Kemp, Aldrich, Guerra, & Schneider, 2001; Northcutt, Berrang, Smith, & Jones, 2003; Oyarzabal, Hawk, Bilgili, Warf, & Kemp, 2004; Stopforth et al., 2007) or not (Goksoy, Kirkan, & Kok, 2004; González-Miret, Escudero-Gilete, & Heredia, 2006; Svobodová, Bořilová, Hulánková, & Steinhauserová, 2012). A systematic literature review commissioned by the EFSA showed that fecal contaminants (*Enterobacteriaceae, E. coli*) changed in the range of -0.05 to +0.83 CFU per ml rinse water or cm^2 after the evisceration step (Barco, Belluco, Roccato, & Ricci, 2014). Reduced microbial counts on the carcass after washing may either be due to a real reduction in microbial contamination, or caused by a more even redistribution of bacteria on a carcass, resulting apparently lower counts per area, while the total amount of bacteria on the carcass remains largely unchanged.

In agreement with our data, other authors also found that air chilling carcasses had a limited effect on bacterial counts on the skin (Allen, Corry, Burton, Whyte, & Mead, 2000; C. James, Vincent, de Andrade Lima, & James, 2006). In contrast to the skin samples, the second chilling phase using dry air resulted in a significant reduction of TVC, *E. coli* and *Enterobacteriaceae* counts in the abdominal cavity, which has also been described by (Allen et al., 2000).

ESBL-producing *Enterobacteriaceae* were detected across all six process steps with an overall prevalence of 6.1 %. This was lower compared to previous research performed in Europe (46 - 88%) (Pacholewicz, Liakopoulos, et al., 2015a; Reich, Atanassova, & Klein, 2013; Schill, Abdulmawjood, Klein, & Reich, 2017), which is probably owed to the quantitative analysis in the present study compared to more sensitive qualitative testing. This is also the most probable reason why none of the samples from the smallest group, organic broilers, were ESBL positive. Alternatively, this observation may be due to the presumably lower use of antibiotics in organic raising systems. However, this second hypothesis is contradicted by the longer lifespan and more frequent exposure to wild birds of broilers in organic raising systems, which would be expected to increase the probability of a broiler being ESBL positive. The $bla_{\text{CTX-M-1}}$ gene has very frequently been identified in multiresistant bacteria originating from the poultry production environment (Casella, Nogueira, Saras, Haenni, & Madec, 2017; Day et al., 2016; Irrgang et al., 2018; Smet et al., 2008). In line with these findings, all ESBL producers identified in this study were positive for $bla_{\text{CTX-M-1}}$.

We found a prevalence of *Campylobacter*-positive carcasses of 16 %, which was considerably lower than what has been described by other authors in Switzerland (29 %) (Althaus, Zweifel, & Stephan, 2017) and in the EU, where a long-term meta-analysis of literature published between 2000 and April 2017 found a prevalence of 33.3% for *Campylobacter* in poultry meat (Gonçalves-Tenório, Silva, Rodrigues, Cadavez, & Gonzales-Barron, 2018). Again, the comparatively low prevalence of *Campylobacter* and ESBL producers in the current study is most likely owed to the quantitative testing compared to the qualitative methods used in other studies. Carcasses originating from the free-range and organic label rearing systems had a higher prevalence of *Campylobacter* compared to conventionally reared broilers. Access of birds to outdoor space, and the considerably longer

fattening period in free-range and organic rearing systems are plausible explanations (Heuer, Pedersen, Andersen, & Madsen, 2001; Kijlstra, Meerburg, & Bos, 2009).

In the present study, 18 broiler carcasses exhibited *Campylobacter* counts above 1000 CFU/g, either after plucking or after evisceration, while no samples from carcasses after the washing or cooling steps exceeded 1000 CFU/g. For comparison, the data published by the EFSA in 2017 (European Food Safety Authority and European Centre for Disease Prevention and Control (EFSA and ECDC), 2018) showed that nearly half of all carcasses exceeded 1000 CFU/g in Spain, while numbers in the UK ranged from 3.7 – 4.8 % of all carcasses at retail level. In 2018, the EU implemented a quantitative process hygiene criterion for *Campylobacter* on broiler carcasses after chilling (Commission Regulation (EU) 2017/1495 of 23 August 2017). To pass as satisfactory, no more than 20 of 50 samples from 10 consecutive samplings may harbor >1000 CFU/g of *Campylobacter*. The allowable number of samples that exceed 1000 CFU/g will be gradually reduced to 15 in 2020 and to 10 in 2025. In contrast to the study performed here, one sample according to the EU regulation consists of a pool of three neck skin samples from one flock. The reason to collect and pool three skin samples from different locations per animal, and therefore go beyond the samples required by the EU regulation, was to obtain a larger and more representative sample of the carcass surface. Hence, even though the sampling of the present study was not done according to the EU regulation, our data suggest that the chilled carcasses produced in this facility would conform with the new EU regulations regarding *Campylobacter*.

Subtyping showed that a single ST predominated in 13 of 15 *Campylobacter* positive flocks. A different ST was prevailing on almost all processing days, indicating that flocks were colonized with a specific ST before entering the slaughterhouse. ST 21 and ST 48 were the most prevalent ST in this study. These sequence types were frequently found in broiler meat or caeca swab samples by other authors (Di Giannatale et al., 2016; Wieczorek, Denis,

Lachtara, & Osek, 2016; G. Zhang, Zhang, Hu, Jiao, & Huang, 2015), while ST 45 predominated in sample from Finnish broilers and human patients for years (Llarena, Huneau, Hakkinen, & Hänninen, 2015).

The use of quinolones to treat bacterial infections in poultry rearing systems has led to frequent isolation of quinolone-resistant bacteria from poultry and their environment (Antunes, Mourão, Campos, & Peixe, 2016; Kaakoush et al., 2015). In our data, 52% of all *Campylobacter* isolates were resistant to ciprofloxacin. Very high prevalences of quinolone resistance have been reported in *C. jejuni* and *C. coli* isolates. For instance, in 2017, 57.7% of human and 68.8 of broiler isolates in the EU were ciprofloxacin resistant (European Food Safety AuthorityEuropean Centre for Disease Prevention and Control, 2019). In other parts of the world, up to 100% of all isolates were ciprofloxacin resistant (Gharbi et al., 2018; Li et al., 2017; Sierra-Arguello et al., 2016; Woźniak-Biel et al., 2018). Given the high prevalence of ciprofloxacin-resistant *Campylobacter* strains, ciprofloxacin has been largely abandoned for the treatment of human infections, with erythromicin being used as a replacement antibiotic to treat human infections (Bolinger & Kathariou, 2017). In our data, 2.5 % of all isolates were resistant to erythromycin, compared to 2.0 % overall in the EU, with large variations between countries and high numbers in Portugal (6.3 % resistant isolates) and Malta (5.7 % resistant isolates) (European Food Safety AuthorityEuropean Centre for Disease Prevention and Control, 2019).

In conclusion, the quantitative microbial slaughter process analysis performed here narrowed down the main source of microbial contamination on the carcass surface to the process between plucking and evisceration, while the body cavities were contaminated during the evisceration step. The subsequent washing succeeded in reducing microbial counts.

5. Declaration of interest

The authors declare no potential conflict of interest.

6. Funding

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8. Figures and table

Figure 1: Mean TVC, *E. coli* and *Enterobacteriaceae* from skin samples (limit of detection: 100 CFU/g)

(a) after plucking, (b) after cloaca excision, (c) after evisceration, (d) after washing, (e) after chiller 1 and (f) after chiller 2; *p<0.05

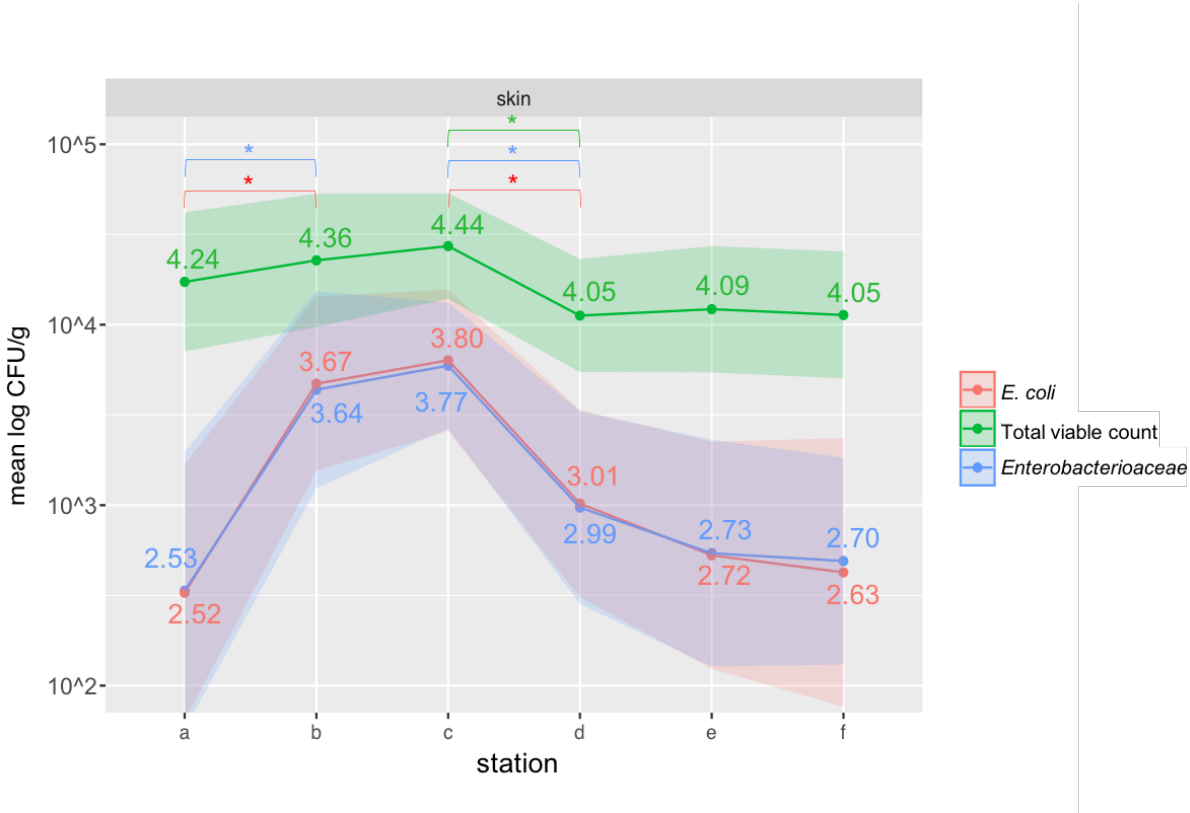

Figure 2: Mean TVC, *E. coli* and *Enterobacteriaceae* from abdominal and thorax (limit of detection: 10 CFU/cm^2)

(a) after plucking, (b) after cloaca excision, (c) after evisceration, (d) after washing, (e) after chiller 1 and (f) after chiller 2; *p<0.05

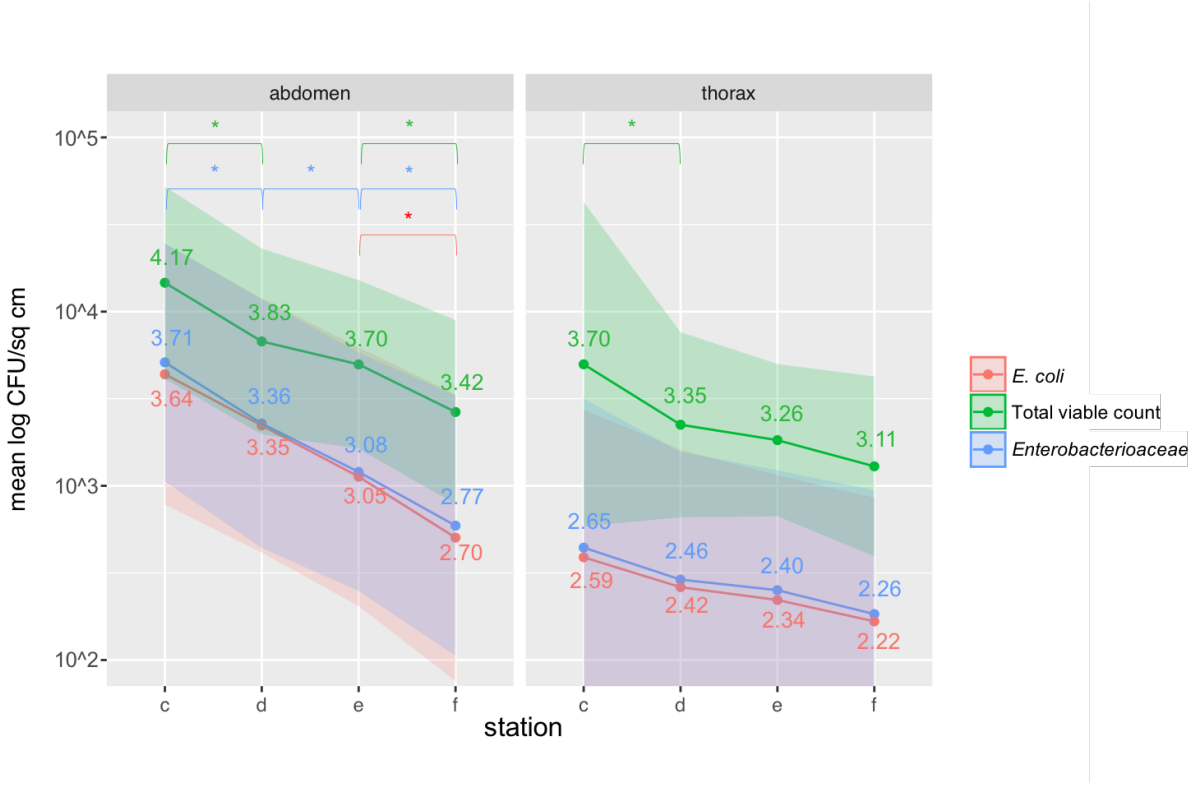

Figure 3: Number of carcasses that were positive for a) *Campylobacter* and b) ESBL. X-axis: production label. Y-axis: no of carcasses. Numbers in bars represent n=carcasses that showed counts above/below the limit of detection (100 CFU/g for skin samples, 10 CFU/cm^2 for thoracic and abdominal swabs).

Figure 3a

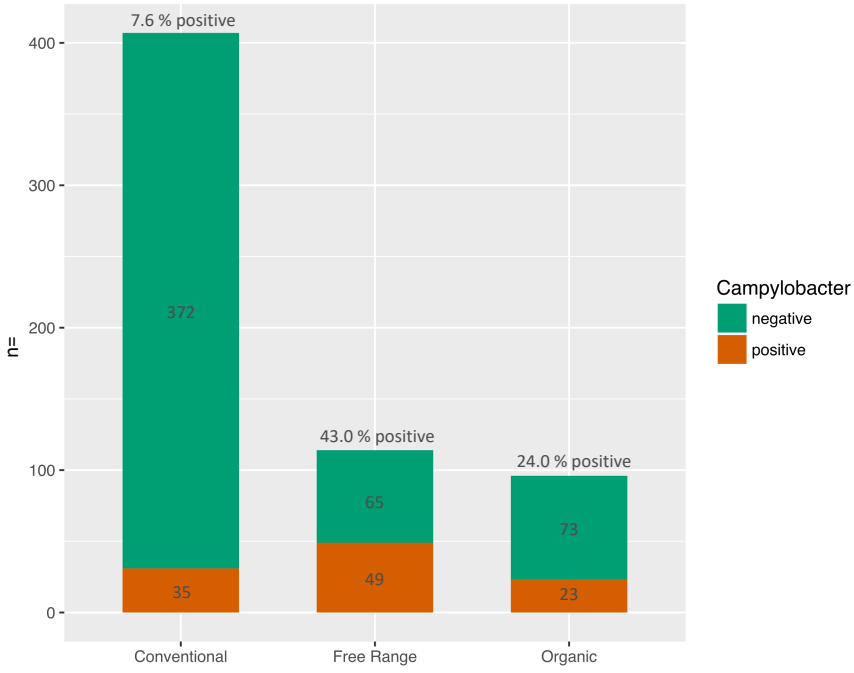

Figure 3b

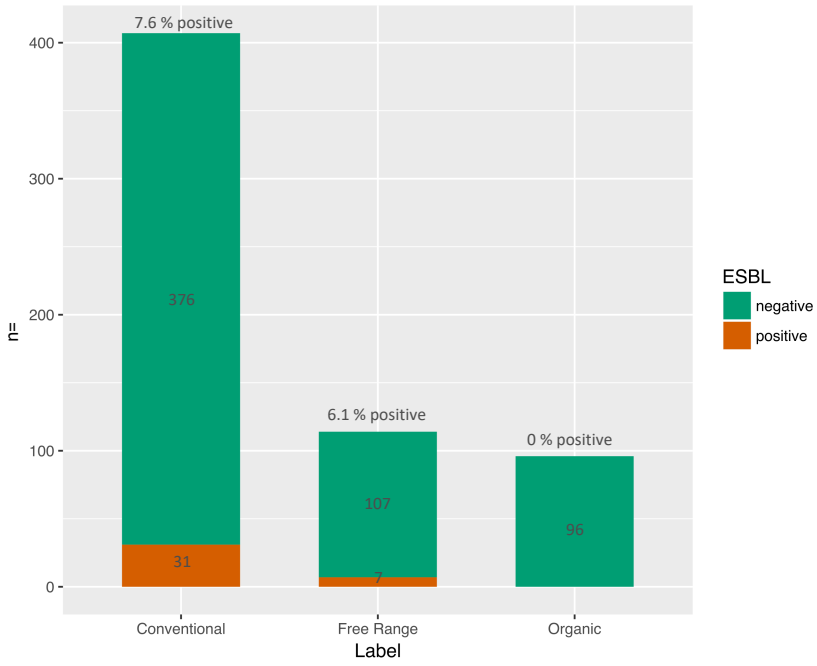

Table 1: Distribution of sequence types among *Campylobacter jejuni* isolates

No of isolate	Sample	Date	Station[1,2]					
			a	b	c	d	e	f
15	thorax	22.02.18			11			
17	abdomen						21	
18	thorax							21
21	skin				11			
21	abdomen				11			
56	skin	01.03.18		51				
57	abdomen				51			
58	abdomen					51		
60	abdomen							51
128	skin	22.03.18		50				
129	skin				50			
129	abdomen				50			
130	abdomen					50		
131	skin						50	
131	abdomen						50	
132	skin							50
134	skin			50				
135	skin				50			
186	thorax	03.04.18						9454
230	skin	10.04.18		11				
236	skin			11				
237	skin				11			
374	skin	01.05.18		9455				
375	skin				122			
375	abdomen				122			
376	abdomen					122		
378	abdomen							9455
381	skin				9455			
381	abdomen				9455			
382	skin					9455		
382	abdomen					9455		
383	abdomen						9455	
453	skin	17.05.18			21			
453	abdomen				21			
454	thorax					21		
455	skin						21	
459	skin				21			
459	abdomen				21			
461	abdomen						21	
494	skin	28.05.18		689				
500	skin			9456				
503	skin						42	
517	skin	30.05.18	48					
518	skin			48				
519	skin				48			
519	abdomen				48			
520	skin					48		
520	abdomen					48		
522	abdomen							48
524	skin			48				
525	skin				48			
525	thorax				48			
526	skin					48		
526	abdomen					48		
528	skin							48
528	abdomen							48
530	skin	04.06.18		51				
531	skin				51			
532	skin				51			
532	abdomen				51			
533	abdomen						1073	
534	skin							173
534	abdomen							51
536	skin			51				
537	thorax				21			
538	thorax					51		
539	skin						51	
539	abdomen						51	
540	Skin							51

542	skin	05.06.18		48				
543	skin				21			
543	abdomen				21			
550	abdomen					48		
551	skin						48	
551	abdomen						21	
555	skin	06.06.18			21			
555	abdomen				21			
556	abdomen					21		
557	abdomen						21	
558	skin							21
558	thorax							21
560	skin			21				
561	skin				21			
561	abdomen				21			
562	skin					21		
562	abdomen					21		
563	skin						21	
563	abdomen						21	
565	skin		21					
567	skin				21			
567	abdomen				21			
568	abdomen					21		
569	abdomen						21	
571	skin	07.06.18	9457					
572	skin			9457				
573	skin				9457			
575	abdomen						9457	
577	skin		9457					
578	skin			9457				
579	skin				9457			
579	abdomen				9457			
580	skin					9457		
580	abdomen					9457		
581	abdomen						9457	
582	abdomen							9457
583	skin	11.06.18	21					
585	skin				21			
586	skin					21		
587	thorax						21	
591	abdomen				21			
592	abdomen					21		
593	skin						21	
608	skin	14.06.18		383				
609	abdomen				383			
611	skin						354	
612	skin							383
612	abdomen							383
614	skin			383				
615	abdomen				383			
617	abdomen						383	

[1]Stations: (a) after plucking, (b) after cloaca excision, (c) after evisceration, (d) after washing, (e) after chiller 1 and (f) after chiller 2
[2]Numbers in colored boxes represent sequence types.

9. Supplementary

Supplementary table 1: Primers used for *C. jejuni* multi locus sequence typing

Gene	Primer	Primer Sequence 5'-3'
aspA	aspA_S3_fw	CCAACTGCAAGATGCTGTACC
	aspA_S6_rev	TTCATTTGCGGTAATACCATC
glnA	glnA_S3_fw	CATGCAATCAATGAAGAAAC
	glnA_S6_rev	TTCCATAAGCTCATATGAAC
gltA	gltA_S3_fw	CTTATATTGATGGAGAAAATGG
	gltA_S6_rev	CCAAAGCGCACCAATACCTG
glyA	glyA_S3_fw	AGCTAATCAAGGTGTTTATGCGG
	glyA_S4_rev	AGGTGATTATCCGTTCCATCGC
	glyA_rev_new[1]	GGACTTCTAATCTCTCCTGGAACG
pgm	pgm_S5_fw	GGTTTTAGATGTGGCTCATG
	pgm_S2_rev	TCCAGAATAGCGAAATAAGG
tkt	tkt_S5_fw	GCTTAGCAGATATTTTAAGTG
	tkt_S6_rev	AAGCCTGCTTGTTCTTTGGC
uncA	uncA_S3_fw	AAAGTACAGTGGCACAAGTGG
	uncA_S4_rev	TGCCTCATCTAAATCACTAGC

[1] for some strains, glyA_S4_rev was replaced by glyA_rev_new

Supplementary table 2: Overview of *bla* genes and resistance profiles of ESBL producing *E. coli* isolates

Isolate	Sample	Species	*bla*-genes	Phylogroup	Antibiotic[1, 2]															
					AM 10	CZ 30	CTX 30	AMC 30	FEP 30	NA 30	CIP 5	SXT	FOS 200	AZM 15	F/M 300	S10	K30	GM 10	C30	Te 30
e29	thorax	*E. coli*	CTX-M-1/TEM-1b	A	r	r	r	i	r	s	s	r	s	s	s	r	r	s	s	s
e29	abdomen	*E. coli*	CTX-M-1/TEM-1b	A	r	r	r	s	i	s	s	r	s	s	s	r	r	s	s	s
f30	thorax	*E. coli*	CTX-M-1/TEM-1b	A	r	r	r	s	i	s	s	r	s	s	s	r	r	s	s	s
f30	abdomen	*E. coli*	CTX-M-1/TEM-1b	A	r	r	r	s	i	s	s	r	s	s	s	r	r	s	s	s
f36	thorax	*E. coli*	CTX-M-1/TEM-1b	A	r	r	r	s	i	s	s	r	s	s	s	r	r	s	s	s
c57	thorax	*E. coli*	CTX-M-1	A	r	r	r	s	i	r	s	s	s	s	s	s	s	s	s	s
c243	thorax	*E. coli*	CTX-M-1	A	r	r	r	s	i	r	s	s	s	r	s	s	s	s	s	s
c243	abdomen	*E. coli*	CTX-M-1	A	r	r	r	s	i	r	s	s	s	s	s	s	s	s	s	s
d244	skin	*E. coli*	CTX-M-1	A	r	r	r	s	i	r	s	s	s	s	s	s	s	s	s	s
d244	thorax	*E. coli*	CTX-M-1	A	r	r	r	s	i	r	s	s	s	s	s	s	s	s	s	s
d244	abdomen	*E. coli*	CTX-M-1	A	r	r	r	s	i	r	s	s	s	s	s	s	s	s	s	s
e245	abdomen	*E. coli*	CTX-M-1	A	r	r	r	s	i	r	s	s	s	s	s	s	s	s	s	s
c249	abdomen	*E. coli*	CTX-M-1	A	r	r	r	s	i	r	s	s	s	s	s	s	s	s	s	s
d250	abdomen	*E. coli*	CTX-M-1	A	r	r	r	s	i	r	s	s	s	s	s	s	s	s	s	s
e251	thorax	*E. coli*	CTX-M-1	A	r	r	r	s	i	r	s	s	s	s	s	s	s	s	s	s
f252	abdomen	*E. coli*	CTX-M-1	A	r	r	r	s	i	r	s	s	s	s	s	s	s	s	s	s
b254	skin	*E. coli*	CTX-M-1	A	r	r	r	s	i	r	s	s	s	s	s	s	s	s	s	s
c255	abdomen	*E. coli*	CTX-M-1	A	r	r	r	s	i	r	s	s	s	s	s	s	s	s	s	s
d256	abdomen	*E. coli*	CTX-M-1	A	r	r	r	s	i	r	i	s	s	s	s	s	s	s	s	s
c273	skin	*E. coli*	CTX-M-1	A	r	r	r	s	r	r	s	s	s	s	s	s	s	s	s	s
d274	abdomen	*E. coli*	CTX-M-1	A	r	r	r	i	i	r	s	s	s	s	s	s	s	s	s	s
e275	thorax	*E. coli*	CTX-M-1	A	r	r	r	s	i	r	s	s	s	s	r	s	s	s	s	s
e275	abdomen	*E. coli*	CTX-M-1	A	r	r	r	s	i	r	s	s	s	s	s	s	s	s	s	s
c279	abdomen	*E. coli*	CTX-M-1	A	r	r	r	s	s	r	s	s	s	s	s	s	s	s	s	s
a295	skin	*E. coli*	CTX-M-1/SHV-12	E	r	r	r	s	s	r	s	s	s	s	s	s	s	s	s	s
c297	abdomen	*E. coli*	CTX-M-1/SHV-12	E	r	r	r	s	i	r	s	s	s	s	s	s	s	s	s	s
f300	abdomen	*E. coli*	CTX-M-1/TEM-1b	D	r	r	r	s	i	r	s	s	s	s	s	s	s	s	s	s
f306	abdomen	*E. coli*	CTX-M-1/SHV-12	E	r	r	r	s	i	r	s	s	s	s	s	s	s	s	s	s
c339	abdomen	*E. coli*	CTX-M-1	A	r	r	r	i	r	r	s	s	s	s	s	s	s	s	s	s
c555	skin	*E. coli*	CTX-M-1	A	r	r	r	s	s	i	s	s	s	s	s	s	s	s	s	s
c555	thorax	*E. coli*	CTX-M-1	A	r	r	r	s	i	i	s	s	s	s	s	s	s	s	s	s
c555	abdomen	*E. coli*	CTX-M-1	A	r	r	s	s	s	i	s	s	s	s	s	s	s	s	s	s
d556	abdomen	*E. coli*	CTX-M-1	A	r	r	r	s	i	r	s	s	s	s	s	s	s	s	s	s
e557	thorax	*E. coli*	CTX-M-1	A	r	r	r	s	i	r	s	s	s	s	s	s	s	s	s	s
e557	abdomen	*E. coli*	CTX-M-1	A	r	r	r	s	i	r	s	s	s	s	s	s	s	s	s	s
f558	thorax	*E. coli*	CTX-M-1	A	r	r	r	s	i	r	s	s	s	s	s	s	s	s	s	s
f558	abdomen	*E. coli*	CTX-M-1	A	r	r	r	s	s	i	s	s	s	s	s	s	s	s	s	s
c561	abdomen	*E. coli*	CTX-M-1	A	r	r	r	s	i	r	s	s	s	s	s	s	s	s	s	s
d562	abdomen	*E. coli*	CTX-M-1	A	r	r	r	s	i	r	s	s	s	s	s	s	s	s	s	s
e563	thorax	*E. coli*	CTX-M-1	A	r	r	r	s	i	i	s	s	s	s	s	s	s	s	s	s
e563	abdomen	*E. coli*	CTX-M-1	A	r	r	r	s	i	i	s	s	s	s	s	s	s	s	s	s
f564	abdomen	*E. coli*	CTX-M-1	A	r	r	r	s	i	i	s	s	r	s	s	s	s	s	s	s
c567	skin	*E. coli*	CTX-M-1	A	r	r	r	s	i	i	s	s	s	s	s	s	s	s	s	s
d568	abdomen	*E. coli*	CTX-M-1	A	r	r	r	s	i	i	s	s	s	s	s	s	s	s	s	s

b584	skin	*E. coli*	CTX-M-1	A	r	r	r	s	s	r	s	s	s	s	s	s	s	s	s	s
c585	skin	*E. coli*	CTX-M-1	A	r	r	r	s	i	r	s	s	s	s	s	s	s	s	s	s
c585	thorax	*E. coli*	CTX-M-1	A	r	r	r	s	i	r	s	s	s	s	s	s	s	s	s	s
c585	abdomen	*E. coli*	CTX-M-1	A	r	r	r	s	i	r	s	s	s	s	s	s	s	s	s	s
f588	skin	*E. coli*	CTX-M-1	A	r	r	r	s	i	r	s	s	s	s	s	s	s	s	s	s
c591	thorax	*E. coli*	CTX-M-1	A	r	r	r	s	i	r	s	s	s	s	s	s	s	s	s	s
f594	abdomen	*E. coli*	CTX-M-1	A	r	r	s	s	s	r	s	s	s	s	s	s	s	s	s	s

[1]Antibiotics used: ampicillin (AM), amoxicillin-clavulanic acid (AMC), azithromycin (AZM), cefazolin (CZ), cefepime (FEP), cefotaxime (CTX), chloramphenicol (C), ciprofloxacin (CIP), fosfomycin (FOS), gentamicin (GM), kanamycin (K), nalidixic acid (NA), nitrofurantoin (F/M), streptomycin (S), sulfamethoxazole with trimethoprim (SXT) and tetracycline (TE). Numbers behind abbreviations indicate concentrations in µg/ml

[2]r=resistant, i=intermediate, s=sensitive

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